Amendments to the Specification:

Please replace the paragraph beginning at page 1, line 16 (which carries over to page 2, line 3) with the following amended paragraph:

The bacterium is endemic in hospitals, and studies have shown that approximately one third of patients receiving antibiotic treatment in acute-care medical wards were ~~colonised~~ colonized by *C. difficile* while in hospital (Kyne, L. , *et al.*, 2002, Clin. Infect. Dis. 34 (3), pp346-53, PMID: 11774082). Of these patients, over half went on to develop CDAD while the remainder were symptomless carriers. CDAD is a major factor in extension of patient hospital stay times, and estimates suggest that the cost of this disease in the US exceeds $1.1 billion per year (Kyne, L., *et al.*, Supra). Patients suffering from CDAD respond well to a treatment which includes a discontinuation of the inciting antibiotic and treatment with either of the antibiotics metronidazole and vancomycin. However, the use of e.g. vancomycin is one of last resort since it is associated with several problems. Not only may it cause nephrotoxicity, ototoxicity, bone marrow toxicity and the red man syndrome, but the problem with this treatment regime is that the CDAD often returns after successful treatment of the initial episode, and this reoccurrence represents a serious clinical problem. Additionally, there is evidence that *C. difficile* is becoming resistant to metronidazole and partially resistant to vancomycin, demonstrating the need for new alternatives in the treatment of CDAD.

Please replace the paragraph beginning at page 4, line 13 with the following amended paragraph:

There are, however, several problems associated with current passive immunotherapy regimes aimed at treating *C. difficile* infections. For example, passive immunotherapy requires that there are survivors of the *C. difficile* infection and patients who have been vaccinated. Each batch of antibody can be different leading to difficulties associated with ~~standardisation~~ standardization and administration of the immunotherapeutic reagent. In addition, the problem of inadvertent

administration to a patient of adventitious agents (e.g HIV, HBV, HCV, or as yet unidentified agents) is a real one, and up-to-date screening of any immunotherapeutic reagent is required. Finally, the strain variability exhibited by *C. difficile* means that a given antibody may only be useful against certain strains of the bacterium and not against others.

Please replace the paragraph beginning at page 4, line 24 (which carries over to page 5, line 13) with the following amended paragraph:

Obviously the techniques involved are somewhat complex, inconvenient, expensive and time consuming. In general they require that an immunogen is isolated from the infecting pathogen and used to generate antibody. Simply isolating the immunogen can be extremely difficult and time consuming, particularly if it comprises carbohydrate or complex non- linear epitopes (i. e. epitopes having secondary, tertiary and/or quaternary structural features) which cannot be ~~synthesised~~ synthesized *in vitro*, and are impossible to isolate and produce as an antigen for use as e.g. a vaccine. The SLPs of *C. difficile* contain a glycoprotein subunit which varies in molecular weight between species. It may be the case that *C. difficile* epitopes are only produced *in vivo* and are not ~~synthesised~~ synthesized *in vitro*, consistent with for example *Neisseria gonorrhoeae* infections (the causal agent of gonorrhea) where specific antigens are only expressed upon infection of a host. Such antigens fall into the general class of cryptoantigens. Furthermore, *C. difficile* may display highly labile antigens which are difficult to work with since during use they simply degrade and the epitope they display is lost. With regard to this range of epitopes/antigens whose identification and/or *in vitro* use is of great difficulty or impossible, the present invention overcomes these disadvantages and provides a solution by providing antibodies whose CDR regions have been generated in response to *C. difficile* epitopes during antibody responses of patients infected with *C. difficile*.

Please replace the paragraph beginning at page 5, line 15 with the following amended paragraph:

In addition, the prior art typically has to attempt to achieve an equivalent of affinity maturation of antibodies by first ~~synthesising~~ synthesizing a set of candidate antibodies specific to an antigen, testing them for their binding characteristics and then modifying the sequences of the candidates in order to ~~optimise~~ optimize the binding. A thorough attempt at affinity maturation (i.e. ~~optimising~~ optimizing antibody binding) can require the synthesis of thousands of different antibodies, which can be costly and time-consuming. Because the antibodies of the present invention are obtained from patients who have either been infected by a pathogen displaying the antigen or who have been vaccinated with an antigen, they have by their very nature and definition already undergone affinity maturation, as is most clearly demonstrated by the sequences of their CDR3 regions of the variable heavy and variable light chains (i.e. the CDR-H3 and CDR-L3 regions).

Please replace the paragraph beginning at page 8, line 13 with the following amended paragraph:

In addition, the prior art use of library systems has a number of significant problems - in particular, some antibodies produced by a library may cause the death of the organism expressing them and therefore they simply cannot be detected. This is not a particular problem when e.g. looking for antibodies specific to cancers, but when one is searching for antibodies specific to an antigen from a pathogen which might be homologous to one produced by the host expression system (e.g. *Escherichia coli*) then important antibodies cannot be expressed. The use of e.g. *E. coli* to express libraries of e.g. human antibodies also suffers from the problem of codon usage - codons used by humans for specific amino acids can frequently not be the optimum ones for the same amino acid in *E. coli* or other host systems. This means that an important antibody might not be expressed (or at least not in sufficient quantities) since the

codons in its sequence are highly inefficient in *E. coli*, resulting in the *E. coli* being unable to read through and express it in full. Codon ~~optimisation~~ <u>optimization</u> of antibody libraries is obviously not an option since the libraries would first have to be sequenced, which defeats the main advantages of using libraries. Since the present invention sequences antibodies directly from a patient, it avoids this problem.

Please replace the paragraph beginning at page 10, line 19 (which carries over to page 11, line 4) with the following amended paragraph:

From the nucleotide sequences determined by the initial sequencing, putative amino acid sequences for the VH and/or VL regions can be determined using standard algorithms and software packages (e.g. see www.mrc-lmb.cam.ac.uk/pubseq/, the Staden package and Gap4 programs; Rodger Staden, David P. Judge and James K. Bonfield. Managing Sequencing Projects in the GAP4 Environment. Introduction to Bioinformatics. A Theoretical and Practical Approach. Eds. Stephen A. Krawetz and David D. Womble. Human Press Inc., Totawa, NJ 07512 (2003); Rodger Staden, David P. Judge and James K. Bonfield. Analysing Sequences Using the Staden Package and EMBOSS. Introduction to Bioinformatics. A Theoretical and Practical Approach. Eds. Stephen A. Krawetz and David D. Womble. Human Press Inc., Totawa, NJ 07512 (2003)). These can be further ~~chracterised~~ <u>characterized</u> to determine the CDR (Complementarity Determining Region) parts of the VH and/or VL sequences, particularly CDR1, CDR2 and CDR3. Methods for determining the putative amino acid sequences and identifying CDR regions are well known and detailed below.

Please replace the paragraph beginning at page 13, line 13 with the following amended paragraph:

The present invention can be used to determine the sequences of antibodies conferring immunity by looking for over-represented VH and/or VL sequences in patients who have overcome

infection. These protective antibodies can be ~~re-synthesised~~ re-synthesized at the genetic level, over-expressed in *E. coli* (or other expression systems) and purified. The resultant purified recombinant antibody can then be administered to patients as a passive immunotherapy. Antibodies can also be ordered from commercial suppliers such as Operon Technologies Inc, USA (www.operon.com) by simply supplying them with the sequence of the antibody to be manufactured.

Please replace the paragraph beginning at page 14, line 4 with the following amended paragraph:

2) By following alterations in the antibody repertoire over the course of *C. difficile* infection. During infection, antibodies undergo a maturation process to improve pathogen binding and this is ~~chracterised~~ characterized by sequence alterations. Also, B cell clonal expansion is more prevalent in the final stages of infection where the infection is cleared. The most frequent antibodies in the repertoire are chosen as candidates for immunotherapy. Following the maturation process the candidate antibody will demonstrate which key amino acid residue alterations improve antigen binding and this information can be used to improve antibody design.

Please replace the paragraph beginning at page 15, line 2 with the following amended paragraph:

Vaccination protects against infection by priming the immune system with pathogen-derived antigen(s). Vaccination is effected by a single or repeated exposures to the pathogen-derived antigen(s) and allows antibody maturation and B cell clonal expansion without the deleterious effects of the full-blown infectious process. T cell involvement is also of great importance in effecting vaccination of patients. The present invention can be used to monitor the ~~immunisation~~ immunization process with experimental *C. difficile* vaccines. Subjects are given the

experimental vaccine and VH and/or VL sequences are amplified from the patient and the antibody repertoire ~~analysed~~ analyzed as described above. Qualitative and quantitative assessment of the vaccination process is possible:

Please replace the paragraph beginning at page 16, line 1 with the following amended paragraph:

Also provided to the present invention is a method of manufacture of a medicament for the treatment of a *C. difficile* infection which produces at least one antigen, comprising the steps of:

(i) performing a method according to the present invention to identify a set of candidate sequences for antibodies specific against at least one antigen produced by *C. difficile*; and

(ii) ~~synthesising~~ synthesizing at least one antibody comprising a said candidate sequence specific against the at least one antigen produced by *C. difficile*.

Please replace the paragraph beginning at page 16, line 20 (which carries over to page 17, line 2) with the following amended paragraph:

Also provided according to the present invention is a method of treatment of an infection of a patient by *C. difficile* which produces at least one antigen, comprising the steps of :

(i) performing a method according to the present invention to identify a set of candidate sequences for antibodies specific against the at least one antigen produced by *C. difficile*;

(ii) ~~synthesising~~ synthesizing at least one antibody comprising a said candidate sequence specific against the at least one antigen produced by *C. difficile*; and

(iii) administering a therapeutically effective quantity of said at least one ~~synthesised~~ synthesized antibody to said patient.

Please replace the paragraph beginning at page 23, line 22 with the following amended paragraph:

Figure 2 shows a schematic depiction of ~~resynthesised~~ resynthesized recombinant antibody gene cassette. VH and VL regions are linked with a glycine serine-rich linker. Each variable domain contains three Complementarity Determining Regions (CDRs) which participate in antigen binding.

Please replace the paragraph beginning at page 26, line 9 with the following amended paragraph:

For this method, human patients with *C. difficile* infection were selected as donors of ~~immunised~~ immunized B cells. The criteria for selection were:

Please replace the paragraph beginning at page 26, line 22 (which carries over to page 27, line 9) with the following amended paragraph:

Peripheral B-cell lymphocytes (PBLs) were collected from infected patient blood samples. For this ~~heparinised~~ heparinized blood was diluted in PBS (20 ml total) and overlaid onto a 15 ml cushion of Ficol Hypaque (Pharmacia; unless stated otherwise, all chemicals and culture media were purchased from Sigma, UK) in a 30 ml centrifuge tube. The PBLs were then collected by centrifugation (400 x g, 5 minutes) and washed in PBS and harvested by centrifugation again. RNA was prepared from PBLs using the QuickPrep mRNA purification kit (Pharmacia) exactly according to the manufacturers instructions. The isolated mRNA was used to prepare cDNA via a reverse transcriptase reaction (Promega cDNA synthesis kit). For this 2μg of mRNA was re-suspended in 16 μL nuclease-free water and heated to 65 °C for 3 minutes (to denature secondary structure) and then immediately chilled on ice for 1 minute. The mRNA was then added to the

following cocktail: 8 μL 25 mM $MgCl_2$, 4 μL dNTP mix (10 mM with respect to each ribonucleotide triphosphate), 1 μL RNAsin 40 u μL^{-1} stock solution, 1.2 μL AMV reverse transcriptase (25 u μL^{-1} stock solution), 6 μL cDNA 10 pmol μL primer (see Figure 1 - cDNA synthesis). The mixture was incubated at 42 °C for 1 hour and then incubated at 100°C for 3 minutes to stop the reaction.

Please replace the paragraph beginning at page 29, line 22 (which carries over to page 30, line 7) with the following amended paragraph:

DNA sequencing reactions were performed using the DYEnamic ET dye terminator cycle sequencing kit (Amersham Pharmacia Biotech). Purified plasmid DNA (0. 5 μg) was mixed with 8 μL DYEnamic ET terminator reagent premix and 1 μL M13 forward or reverse primer (5 μM) in a total reaction volume of 20 μL. Thermal cycling was then performed using a GeneAmp PCR system 9700 (Perkin Elmer) with the following parameters : 95°C, 20 seconds; 50°C, 15 seconds; 60°C, 1 minute; 30 cycles). Reactions were performed using 96 well format non-skirted ELISA plates (AB Gene). Unincorporated dye terminator ~~were~~ was removed using precipitation. For this, ethanol samples were mixed with 2 μL 7.5 M ammonium acetate and 55 μL of 100 % ethanol and centrifuged at 3000 g for 30 minutes. DNA pellets were washed with 70% ethanol and re-suspended in 20 μL loading solution. Reactions were sequenced using a MegaBACE 1000 DNA sequences (Amersham Pharmacia Biotech) following the manufacturers instructions (2 kV injection voltage for 30 s with electrophoresis at 6 kV for 200 minutes). Chromatograms were exported using the .scf file format for finishing and archiving.

Please replace the paragraph beginning at page 33, line 8 with the following amended paragraph:

Once dominant antibody sequences have been ~~recognised~~ recognized in a given repertoire, the information can be used to infer the presence of CDRs and frameworks that confer immunity.

Selected VH and/or VL sequences can be identified and their gene sequences ~~resnynthesised~~ resynthesized using synthetic oligonucleotides. This is important as it also allows the human-derived gene sequence to be ~~codon-optimised~~ codon-optimized for *E. coli* in order to improve protein expression. Dominant VH and/or VL sequences can be spliced together using a spacer (linker) sequence (Figure 2). This gene cassette, termed a scFv, can be resynthesized to include terminal *Nde*I and *Not*I restriction sites for cloning into expression vector pET29b (Novagen). ScFv DNA can be cut with *Nde*I (cuts in VH) or *Not*I (cuts in VL) using the following reaction: 40 μl DNA (4 μg), 10 μl of Restriction enzyme buffer D (Promega), 47 μl water and 2 μl Ndel and NotI enzyme (10 u μl^{-1}; Promega) with digestion for 4 h at 37°C. DNA can be then fractionated on 0.7% agarose TAE gels and the digested DNA excised from the gel and purified from the agarose slice using the Geneclean II kit (Bio 101) exactly according to the manufacturers instruction. pET29b vector DNA can be cut with *Nde*I and *Not*I as described above. 1 μg vector can be mixed with 1 μg restricted VL DNA resuspended in 8.5 μl water and ligated by addition of 1 μl 10 x ligation buffer (Boehringer Mannheim) and 0.5 μl DNA ligase (3 u μl^{-1}; Boehringer Mannheim), followed by ligation overnight at 14°C.

Please replace the paragraph beginning at page 35, line 5 with the following amended paragraph:

For refolding, 200 ml solution of 2% (w/v) N-lauryl sarcosine (NLS) in 50 mM Tris HCI pH 9.0 can be prepared as follows. 4 g NLS can be ~~solubilised~~ solubilized in 100 ml water with stirring. 10 ml of 1M Tris pH 9.0 stock solution can be added and made up to 195 ml with water. 5 ml of inclusion body slurry can be added and stirred vigorously for 30 minutes at room temperature.

Please replace the paragraph beginning at page 35, line 11 with the following amended paragraph:

$CuCl_2$ can be added to a concentration of 100 μM. This serves as a catalyst for oxidation. The refolding reaction can be transferred to 4°C and stirred vigorously for 2 days to promote aeration. The refolding reaction can be vacuum filtered through a 0.44 μM vacucup bottle top filter unit (90 mm diameter, Gellman Sciences) and the filtrate transferred to a Pellicon Labscale TFF system fitted with PLGC10 membrane unit (Millipore). The reaction can be concentrated to 25 ml using tangential flow, discarding the permeate (the scFv is ~~localised~~ localized to the retentate). The solution can be diafiltered against 40 x turn-over volumes (1 L) of 10 mM ammonium acetate (AAT) pH 9.0. Finally, the volume of the antibody can be diluted to 50 ml using 10 mM AAT pH 9.0. The buffer exchanged antibody can be stored for 2 hours at 4°C. The typical protein content was 1- 2 mg ml^{-1} with a yield of up to 50 mg per litre.

Please replace the paragraph beginning at page 45, line 20 (which carries over to page 46, line 2) with the following amended paragraph:

The presence of a large number of a particular VH CDR3 sequence in a patient with a *C. difficile* infection indicates that VH as part of an scFv may be protective against the organism. CDR3 sequences that are shared by more than one patient indicate specific *C. difficile* sequences. It should also be noted that sequences with a high degree of homology often due to somatic mutation within the patient may also represent important sequences with similar but subtly different properties. Analysis of the CDR3 sequences of the VH from our individuals has ~~lead~~ led to the identification of 17 potentially protective VH's. These VH CDR3 sequences correspond to SEQ ID NOs : 30, 79, 28, 91, 41, 39, 38, 27, 36, 36, 40, 37, 90, 29, 44, 45, and 43.